IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	:	10/583,369
For	:	DELIVERY OF DNA OR RNA VIA GAP JUNCTIONS FROM HOST CELLS TO TARGET CELLS AND A CELL-BASED DELIVERY SYSTEM FOR ANTISENSE OR SIRNA
Applicant	:	Brink et al.
Filed	:	May 24, 2007
Art Unit	:	1635
Conf. No.	:	3149
Examiner	:	Gibbs, Terra C.

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

I, Dr. Ira S. Cohen, do hereby declare:

1. I am a Professor of Physiology & Biophysics and Professor of Medicine at The Institute of Molecular Cardiology at SUNY Stony Brook.

2. For the last 36 years, I have devoted my research efforts to basic cardiovascular research. My curriculum vitae is attached as Exhibit A.

3. I am co-inventor of the subject matter disclosed and currently claimed in the above-identified patent application ("the '369 application"). The invention disclosed in the '369 application relates to a method of delivering an oligonucleotide into a target cell comprising: a) introducing the oligonucleotide or a plasmid expressing the oligonucleotide into a donor cell in vitro; and b) contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction channel whereby the oligonucleotide is delivered into the target cell from the donor cell by traversing the gap junction.

4. I have reviewed the '369 application. I have also reviewed the latest Office Action and the art cited therein. I understand that claims 1-5, 7-9, 11, 12, and 20-23 have been rejected by the Examiner.

5. I understand that the Examiner alleges that claims 1-5, 7-9, 11, 12, and 20-23 are obvious under 35 U.S.C. 103(a) over Rosenthal et al. (Biochimie, 1995 Vol. 77:439-443), as evidenced by Salomon et al. (Journal of Investigational Dermatology, 1994 Vol. 103(2), Abstract only), in view of Hammond et al. (Nature Reviews. Genetics, 2001 Vol. 2:110-119).

6. I understand that the Examiner also alleges that claims 1-5, 7-9, 11, 12, and 20-23 are obvious under 35 U.S.C. 103(a) over Giampuzzi et al. (Journal of Biological Chemistry, 2001 Vol. 276, No:31:29226-29232), as evidenced by Valiunas et al. (Journal of Physiology, 2005 Vol:2:459-468, submitted and made of record on July 6, 2009), in view of Hammond et al. (Nature Reviews. Genetics, 2001 Vol. 2:110-119).

7. The present claims are directed to a method for delivering an oligonucleotide to a target cell by first introducing the oligonucleotide or a plasmid expressing an oligonucleotide into a donor cell in vitro and second contacting the target cell with the donor cell under conditions permitting the donor cell to form a gap junction channel composed of connexin 43 with the target cell, whereby the oligonucleotide is delivered into the target cell from the donor cell by traversing the gap junction wherein the gap junction is composed of connexin 43 and wherein the oligonucleotide is 12 to 24 nucleotides in length.

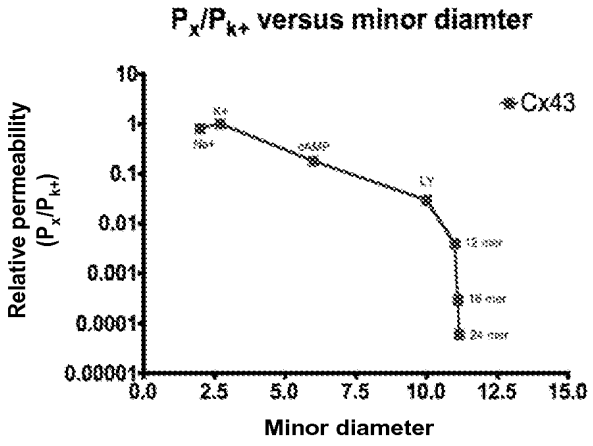
I. The Antisense Oligonucleotides Disclosed in Rosenthal et al. and Giampuzzi et al. Would Not Traverse a Gap Junction

8. I understand that the Office Action acknowledges that "Rosenthal et al. do not explicitly state that the oligonucleotide is delivered to the target cell from the donor cell by traversing the gap junction" (Office Action dated Oct. 28, 2009 at p. 5). Rather, the Office takes the position that Rosenthal et al. implicitly teach the claimed method stating that the Office is "interpreting that the oligonucleotide is delivered to the target cell from the donor cell by traversing the gap junction, absent evidence to the contrary" (Office Action dated Oct. 28, 2009 at p. 5). The Office further states that "it falls to Applicant to determine and

provide evidence that the oligonucleotide taught by Rosenthal et al. is or is not delivered into the target cell from the donor cell by traversing the gap junction as instantly claimed." (Office Action dated Oct. 28, 2009 at p. 6).

9. I understand that the Office also acknowledges that "Giampuzzi et al. do not explicitly state that the oligonucleotide is delivered to the target cell from the donor cell by traversing the gap junction" (Office Action dated Oct. 28, 2009 at p. 10). Rather the Office Action alleges that Giampuzzi et al. implicitly teach the claimed methods stating that the Office is "interpreting that the oligonucleotide is delivered to the target cell from the donor cell by traversing the gap junction, absent evidence to the contrary." (Office Action dated Oct. 28, 2009 at p. 10).

10. Our data shows that oligonucleotides that are 12, 16 and 24 nucleotides in length (single and double stranded) can traverse a gap junction and further shows that the permeation of single-stranded oligonucleotides through gap junctions decreases as a function of the length of oligonucleotide (see Figure 2 of the '369 application). Additional data demonstrates this relationship between length and gap junction permeability (see below figure).



The above figure compares the relative permeability of single-stranded morpholino oligonucleotides through gap junctions composed of connexin 43 in hMSCs, HEK293 and HeLa transfected with Cx43 (methods as described in Valiunas et al.; Valiunas et al. *J. Physiol.* (2009) **587**(21): 5211-5226; and Kanaporis et al. *J. Gen. Physiol.* (2008) **131**(4):293-305). Also shown are the relative permeability of sodium (Na^+) cyclic AMP (cAMP) and lucifer yellow (LY). The permeabilities are relative to that of potassium (K^+) and the relative permeabilities are displayed in the figure on a log scale. Thus, an

oligonucleotide that is 12 nucleotides in length (12 mer) is over 100 times less able to permeate a gap junction than potassium. The 24 mer is over 10,000 times less able to permeate a gap junction than potassium and nearly 100 times less able to permeate a gap junction than the 12 mer. In other words, doubling the length of the oligonucleotide from 12 to 24 nucleotides decreased the permeability of the oligonucleotide by nearly 100 times. If this relationship is extrapolated to oligonucleotides of 1000 nucleotides in length, they would be around $1x10^{83}$ times less permeable than the 12 mer. Therefore, from our data it appears that oligonucleotides of 1000 nucleotides (or more) simply will not traverse a gap junction composed of connexin 43.

11. The PADPRP antisense RNA expressed in keratinocytes by Rosenthal et al. consisted of the entire human PADPRP translated and untranslated regions in reverse orientation. According to the NCBI web site, the coding sequence alone for this gene is 3045 nucleotides and the mRNA is 3984 nucleotides not including the poly-A tail (accession number NM_001618). Thus, the cDNA for PADPRP (including translated and untranslated regions) was over 3,900 nucleotides in length. The antisense RNA generated from cloning this cDNA in reverse orientation would be over 3,900 nucleotides in length and single-stranded.

12. As described above in paragraph 10, our data indicates that an antisense RNA that is 3,900 nucleotides in length (which is about 162 times longer than the 24 nucleotides in length oligonucleotides disclosed the '369 application) would not permeate a gap junction. Moreover, such a long oligonucleotide would have secondary structure that could further preclude permeation through a gap junction. Thus, even if the reconstituted skin formed with the keratinocytes engineered to express the antisense RNA were able to form gap junctions composed of connexin 43 with the nude mouse cells, the PADPRP antisense RNA would not traverse the gap junctions.

13. The LOX antisense RNA expressed in NRK-49F cells by Giampuzzi et al. contained the fragment from -33 to +985 of the mouse LOX cDNA in antisense orientation. Thus, this LOX antisense RNA was about 1,018 nucleotides in length and single-stranded.

14. As described above in paragraph 10, our data indicates that an antisense RNA of about 1,018 nucleotides in length will not traverse a gap junction. Moreover, such a long

oligonucleotide would have secondary structure that could further preclude permeation through a gap junction. Thus, even if the injected NRK-49F cells expressing the antisense LOX could form gap junctions with the nude mouse cells, the LOX antisense RNA would not traverse the gap junctions.

II. One of Skill in the Art Would Not Have Had Motivation to Substitute siRNA Oligonucleotides for the Antisense Oligonucleotides Disclosed in Rosenthal et al. and Giampuzzi et al.

15. At the time the '369 application was filed one of skill in the art would not have been motivated to substitute siRNA oligonucleotides for the antisense oligonucleotides disclosed in Rosenthal et al. and Giampuzzi et al. One of skill in the art would not have expected that oligonucleotides that are 12 to 24 nucleotides in length (including siRNA) would traverse a gap junction (*see* Weber et al., and Simpson et al., discussed below).

16. At the time of filing the '369 application it was believed by those of ordinary skill in the art that gap junctions would generally not allow molecules larger than about 1 kDa to pass and that molecules 1.9 kDa or larger would not permeate a gap junction channel. For example, Simpson et al. (Science, Vol. 21:294-296 (1977); Exhibit B) state "molecules of 1900 daltons or greater do not pass" through gap junction channels. In addition, Weber et al. (Biophysical Journal, Vol. 87:958-973 (2004); Exhibit C) state "Gap junctions (GJ) are the only known intracellular channels that allow the direct exchange of molecules up to ~1 kDa in molecular mass between cells." An oligonucleotide that is about 1 kDa is about 2 to 3 nucleotides in length and the 1.9 kDa cutoff described by Simpson translate to an oligonucleotide of about 5 to 6 nucleotides in length. As explained below, oligonucleotides 12 to 24 nucleotides in length (including siRNAs) are substantially larger than the 1.9 kDa limit for traversing a gap junction described in the art prior to the filing of the '369 application.

17. The molecular weight of a single-stranded, 12 nucleotide long oligonucleotide ranges from about 3.4 kDa to about 4.1 kDa depending on the nucleotide composition and whether the oligonucleotide is made of ribonucleotides or deoxyribonucleotides. Double-stranded oligonucleotides that are 12 base pairs in length will be approximately twice this molecular weight (*i.e.*, from about 7.2 to about 7.8 kDa) depending on the nucleotide composition and whether the oligonucleotide is made of ribonucleotides or deoxyribonucleotides. Longer oligonucleotides will have even higher molecular weights.

18. The molecular weight of an oligonucleotide that is 12 to 24 nucleotides in length is substantially larger than the molecular weight of a molecule that one of ordinary skill at in the art the time of filing the '369 application would have considered capable of traversing a gap junction. Therefore, due to the molecular weight of oligonucleotides from 12 to 24 nucleotides in length, one of ordinary skill in the art at the time the '369 application was filed, would not have expected such oligonucleotides to traverse a gap junction.

19. I, Dr. Ira S. Cohen, declare under penalty of perjury that the above statements are true and correct to the best of my knowledge, information, and belief. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: April 27, 2010

Ira S Cohen

Ira S. Cohen, M.D.